

## Comparison of protein detection and traditional microbiological methods for monitoring food contact surface hygiene in tourist facilities: a 3-years survey

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### Original Research Article

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**Abstract:** Food safety is a major concern for the tourist industry, and food contact surfaces play an important role in transmission of pathogens to food by cross contamination. Different methodologies are used for sanitary monitoring. The aim of this study was to compare two different techniques for hygiene monitoring in hotels. In this survey, 4,628 samples from 279 different hotels were investigated by protein detection and microbiological techniques. Correlation between the two methods was statistically significant ( $\chi^2 = 195.057$ ;  $p < 0.001$ ). Overall, sanitary conditions of the food contact surfaces in our hotels are satisfactory. Differences were detected among the different types of surfaces. The appropriateness of protein monitoring for cleanliness assessment in these facilities is discussed.

**Keywords:** food contact surface; protein detection; hygiene monitoring; rapid testing; tourist, hotel, HACCP.

### INTRODUCTION

Food safety is a major concern for the tourist industry, being food poisoning one of the problems hoteliers are most afraid of. All food businesses, including hotels, are required to operate food safety management procedures based on Hazard Analysis and Critical Control Point (HACCP) principles (EU Directive EC No. 852/2004). Food handler hygiene standards, facilities' cleaning plan, supplier evaluation, temperature monitoring and pest control are among the parameters to be included in these programmes.

Food contact surfaces play an important role in transmission of pathogens to food by cross contamination. Although in most of cases food-borne illness are attributed to inappropriate cooking, temperature abuse and/or contaminated raw ingredients; cross-contamination via contact surfaces has also been identified as a significant factor [1, 2].

Some bacteria, including food pathogens, are able to attach onto surfaces as biofilms [3, 4]. Biofilm formation allows bacteria to persist in adverse conditions and become a continuous contamination source of spoilage bacteria and pathogens in the food processing facilities [3, 5, 6]. Due to this adaptation, biofilm bacteria are less sensitive to cleaning and disinfection than planctonic forms [7].

Previous studies have compared the relative sensitivities of traditional methods, based on microbiological analysis, and recently developed methods like ATP bioluminescence or protein detection. These studies have been performed in controlled laboratory conditions [8], as well as the industry [9, 10] and hospital [11, 12] settings. However,

data from touristic institutions remains scarce. The aim of this study was to evaluate the results of protein and microbiological methods used in hotel kitchens. This will allow us to compare both methods and determine the appropriateness of their use in these facilities. Moreover, will assess the cleaning and sanitizing procedures, and develop a baseline for future comparisons, particularly after implementation of new procedures or revision of the ones applied nowadays.

### MATERIAL AND METHODS

#### Sampling procedures

As part of a larger investigation, facilities evaluated in this study were located in Spanish touristic regions. Hotels were visited without prior notice during a three-year survey, from 2007 to 2009. Food contact

surfaces considered clean by the establishment were randomly selected at the moment of the visit. Different researchers were responsible for sampling and analyzing through the study. Initial training sessions were achieved by the microbiologist, and different internal controls were introduced from beginning to end of the study to ensure homogeneity. Written working instructions were available to all people involved in data compilation.

### Protein analysis

The sanitary conditions of the food contact surfaces were analyzed by a protein detection approach. For this purpose, the colorimetric test MICROKIT®-KIT PRO-5S (Microkit, Madrid, Spain) for surface hygiene control was used following manufacturer's instructions. For each sample, a 100 cm<sup>2</sup> area was delimited with a sterile stainless template (10 × 10 cm), and then analyzed in situ. Swabbed samples were introduced into a tube containing the reactive, and a positive blue signal indicated the presence of proteins.

### Microbiological analysis

Microbiological levels were also analyzed. Aerobic count was determined by 25 cm<sup>2</sup> gridded contact plates. Samples were taken by contacting TLHTh-containing Plate Count Agar (Sharlab, Barcelona, Spain) onto the analyzed surface for 10 s, refrigerated and transported to the laboratory in a mobile incubator. On arrival, samples were incubated at 31 °C for 48 h, and further total aerobic count was calculated.

### Interpretation of results

A surface was considered dirty when protein was detected. As for the microbiological analysis, in the present survey a surface with >10 CFU cm<sup>-2</sup> was considered as dirty.

Statistical analyses were calculated with the SPSS statistical package for Windows (release 10.0.6). The  $\chi^2$  test was used to determine whether there was a correlation between the results obtained with the two monitoring methods.

### RESULTS

A total of 4,628 samples from 279 different hotels were analyzed throughout the survey. Hygienic conditions of the surfaces analyzed by the two methods are shown in Table-1. Correlation between the two methods was statistically significant ( $\chi^2 = 195.057$ ;  $p < 0.001$ ). The number of clean surfaces was clearly higher than that for dirty samples in both techniques. However, data showed that the ratio of dirty surfaces was lower for the protein technique than the traditional based on microbial detection (Table-1 and Figure-1). When individually analyzed, 77% of the results estimated clean by protein absence were confirmed by the microbiological analysis, whereas 52.6% of positives in the protein analysis were corroborated as dirty by the microbial levels. On the other hand, 93.4% of the results evaluated as clean based on the microbial levels were also confirmed as clean by the protein technique, while 79.1% of the results considered dirty by the microbiological levels were evaluated as clean by the protein result.

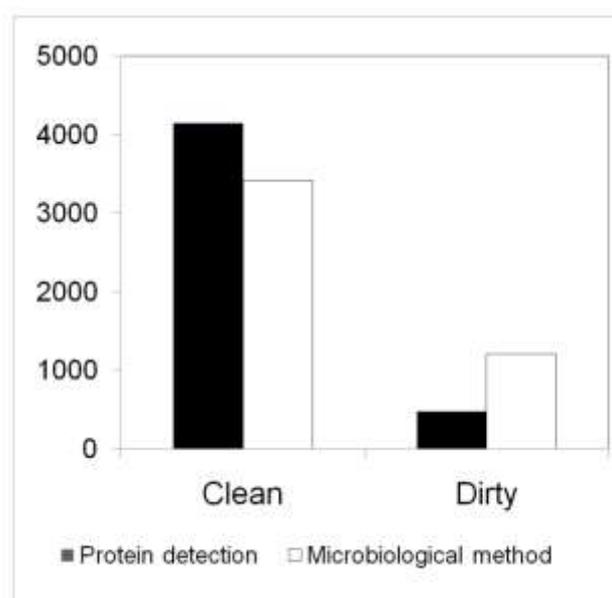


Fig-1: Quantity of clean and dirty surfaces as determined by the two methods

**Table-1: Comparison of results of protein and microbiological contact methods, according to set clean and dirty values**

		Microbiological contact method (MCM)		Total	
		Clean	Dirty		
Protein method	Clean	<i>n</i>	3,194	955	4,149
		% within Protein	77.0%	23.0%	100.0%
		% within MCM	93.4%	79.1%	89.6%
	Dirty	<i>n</i>	227	252	479
		% within Protein	47.4%	52.6%	100.0%
		% within MCM	6.6%	20.9%	10.4%
Total	<i>n</i>	3,421	1,207	4,628	
	% within Protein	73.9%	26.1%	100.0%	
	% within MCM	100.0%	100.0%	100.0%	

$$\chi^2 = 195.057; df = 1; p < 0.001$$

Overall, only 479 (10.35%) of the analyzed surfaces were dirty by the protein method, while microbial levels averaged 3.96 CFU cm<sup>-2</sup> (Table-2). The examined food contact surfaces were classified within four groups. Based on the protein results, chopping and mincing machines presented the worst hygienic

conditions, followed by knives and cutting boards. Finally, kitchenware was clearly cleaner than the rest. Nonetheless, cutting boards showed the higher microbiological counts, followed by chopping and mincing machines and knives. Again, the best hygienic conditions were associated to kitchenware.

**Table-2: Protein presence and mean CFU cm<sup>-2</sup> in food contact surfaces.**

Surface	n	No. (%) of protein positive samples	Mean ± SD (CFU cm <sup>-2</sup> )
Kitchenware	2,169	114 (5.26%)	2.34 ± 3.96
Knives	906	144 (15.89%)	3.48 ± 4.68
Chopping/mincing machines	113	23 (20.35%)	4.31 ± 5.02
Cutting boards	1,440	198 (13.75%)	6.66 ± 5.50
Total	4,628	478 (10.35%)	3.96 ± 5.02

## DISCUSSION

In this study we have surveyed the hygienic conditions of food contact surfaces by two approaches: protein detection and microbiological analysis. The correlation between the two methods was statistically significant. Besides, our 74.2% of agreement is clearly higher than the 57.8% reported for cleaned surfaces in the food industry by [9]. Therefore, the rapid protein method seems generally appropriate for surfaces monitoring in hotel kitchens. This is an important point because, opposite to industry, these facilities lack laboratories and specialized staff for microbial monitoring. Moreover, the protein method is immediate and inexpensive, even cheaper than ATP bioluminescence, with more expensive reagents and the requirement of a luminometer. However, as described in [9], an important number of surfaces that fails in the microbial method pass in the protein analysis. For this reason, although the protein approach may be used in a routinely basis, periodic microbial cultures should be performed to ensure an accurate assessment.

Traditionally, hygienic conditions have been evaluated by microbial counts, either by swabbing or by the contact technique. However, there are no standards for food contact surface cleaning, and different levels have been used in several studies. In the present study the standard was set on 10 CFU cm<sup>-2</sup> [11, 13-15]. In

general, sanitary conditions of the food contact surfaces in Spanish hotels are satisfactory, as the average CFU cm<sup>-2</sup> is clearly under the standard. In fact, microbial levels are lower than those reported for child care facilities [16], living-assisted [17] and processing plants [18]. Protein results also support the good surface cleanliness, as only about 10% of the studied surfaces were considered dirty. In an industrial trial, protein was detected in about 30% of surfaces sampled after they were cleaned [9]. Different factors may play a role in these differences. Personal hygiene, appropriate kitchen design and cleaning and sanitizing procedures adapted to public facilities are described as main points to reduce food contact surfaces contamination [19]. Probably the control systems operating in our installations, from good manufacturer practices to full HACCP systems, are a key tool to ensure these satisfactory levels.

Different types of food contact surfaces have been compared, including kitchenware, knives, chopping/mincing machines and cutting boards. The last group shows the higher microbiological levels, although the mean CFU value still remains under the standard. Nevertheless, nearly 50% of the analyzed cutting boards presented inappropriate levels. These results are similar to those obtained in living-assisted and child-care centres [17, 20]. These high values often

corresponded to scored and damaged boards, therefore making the cleaning and disinfection of the surface difficult. That was also observed in food premises in the United Kingdom [21]. Probably, discrepancies observed between protein and microbial results for these surfaces are, at least partially, due to this fact, as noted by [13]. Agreement between the protein and microbiological results is observed for the other surfaces: kitchenware shows excellent sanitary conditions. In fact, when microbiological levels are compared to those obtained for similar surfaces in living-assisted facilities, the results obtained for hotels in our study are more than seven times lower [17]. Knives present intermediate conditions and finally machinery presents the worst conditions.

## CONCLUSION

In summary, we have compared a rapid and a traditional method to survey the cleaning and sanitizing procedures applied to food contact surfaces in touristic services. Correlation between both methods indicates that protein monitoring may be used for routinely monitoring, but does not eliminate the need for periodic microbial controls. Overall, surfaces in our hotels meet the hygiene standards. Moreover, when compared to other sectors, surfaces present better sanitary conditions than those reported for child care, living-facilities and domestic kitchen. The important amount of samples tested and the large period of time covered makes this report a representative picture of the actual situation. Besides, it sets a basis to develop strategies to improve the sanitary conditions in our hotels and avoid undesirable food poisoning episodes that may occur among tourists.

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